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RAW SEQUENCE LISTING

PATENT APPLICATION: US/10/017,145

DATE: 01/10/2002

TIME: 15:51:00

Input Set : A:\ES.txt

Output Set: N:\CRF3\01102002\J017145.raw

ENTERED

3 <110> APPLICANT: Brookhaven Science Associates
4 Shanklin, John
6 <120> TITLE OF INVENTION: Mutant Fatty Acid Desaturase and Methods for Directed Mutagenesis
8 <130> FILE REFERENCE: CIP of 09/328,550 filed June 9, 1999; which was a CIP of 09/233,856 filed
W--> 9 January 19, 1999
C--> 11 <140> CURRENT APPLICATION NUMBER: US/10/017,145
C--> 11 <141> CURRENT FILING DATE: 2001-12-14
11 <150> PRIOR APPLICATION NUMBER: 09/328,550
12 <151> PRIOR FILING DATE: 1999-06-09
14 <160> NUMBER OF SEQ ID NOS: 13
16 <170> SOFTWARE: PatentIn version 3.1
18 <210> SEQ ID NO: 1
19 <211> LENGTH: 363
20 <212> TYPE: PRT
21 <213> ORGANISM: Ricinus communis
23 <220> FEATURE:
24 <221> NAME/KEY: misc_feature
25 <223> OTHER INFORMATION: ricinus communis delta 9 18:0 Acyl ACP Desaturase
28 <400> SEQUENCE: 1
30 Ala Ser Thr Leu Lys Ser Gly Ser Lys Glu Val Glu Asn Leu Lys Lys
31 1 5 10 15
34 Pro Phe Met Pro Pro Arg Glu Val His Val Gln Val Thr His Ser Met
35 20 25 30
38 Pro Pro Gln Lys Ile Glu Ile Phe Lys Ser Leu Asp Asn Trp Ala Glu
39 35 40 45
42 Glu Asn Ile Leu Val His Leu Lys Pro Val Glu Lys Cys Trp Gln Pro
43 50 55 60
46 Gln Asp Phe Leu Pro Asp Pro Ala Ser Asp Gly Phe Asp Glu Gln Val
47 65 70 75 80
50 Arg Glu Leu Arg Glu Arg Ala Lys Glu Ile Pro Asp Asp Tyr Phe Val
51 85 90 95
54 Val Leu Val Gly Asp Met Ile Thr Glu Glu Ala Leu Pro Thr Tyr Gln
55 100 105 110
58 Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala Ser
59 115 120 125
62 Pro Thr Ser Trp Ala Ile Trp Thr Arg Ala Trp Thr Ala Glu Glu Asn
63 130 135 140
66 Arg His Gly Asp Leu Leu Asn Lys Tyr Leu Tyr Leu Ser Gly Arg Val
67 145 150 155 160
70 Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser Gly
71 165 170 175
74 Met Asp Pro Arg Thr Glu Asn Ser Pro Tyr Leu Gly Phe Ile Tyr Thr
75 180 185 190
78 Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala Arg
79 195 200 205
82 Gln Ala Lys Glu His Gly Asp Ile Lys Leu Ala Gln Ile Cys Gly Thr

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```

86 Ile Ala Ala Asp Glu Lys Arg His Glu Thr Ala Tyr Thr Lys Ile Val
87 225                230                235                240
90 Glu Lys Leu Phe Glu Ile Asp Pro Asp Gly Thr Val Leu Ala Phe Ala
91                245                250                255
94 Asp Met Met Arg Lys Lys Ile Ser Met Pro Ala His Leu Met Tyr Asp
95                260                265                270
98 Gly Arg Asp Asp Asn Leu Phe Asp His Phe Ser Ala Val Ala Gln Arg
99                275                280                285
102 Leu Gly Val Tyr Thr Ala Lys Asp Tyr Ala Asp Ile Leu Glu Phe Leu
103                290                295                300
106 Val Gly Arg Trp Lys Val Asp Lys Leu Thr Gly Leu Ser Ala Glu Gly
107 305                310                315                320
110 Gln Lys Ala Gln Asp Tyr Val Cys Arg Leu Pro Pro Arg Ile Arg Arg
111                325                330                335
114 Leu Glu Glu Arg Ala Gln Gly Arg Ala Lys Glu Ala Pro Thr Met Pro
115                340                345                350
118 Phe Ser Trp Ile Phe Asp Arg Gln Val Lys Leu
119                355                360

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122 <210> SEQ ID NO: 2

123 <211> LENGTH: 1092

124 <212> TYPE: DNA

125 <213> ORGANISM: Ricinus communis

127 <220> FEATURE:

128 <221> NAME/KEY: misc_feature

129 <223> OTHER INFORMATION: residues 138 to 1239 of open reading frame

132 <400> SEQUENCE: 2

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133 gcctctaccc tcaagtctgg ttctaaggaa gttgagaatc tcaagaagcc tttcatgcct      60
135 cctcgggagg tacatgttca ggttacccat tctatgccac cccaaaagat tgagatcttt      120
137 aaatccctag acaattgggc tgaggagaac attctggttc atctgaagcc agttgagaaa      180
139 tgttggcaac cgcaggattt ttgcccagat ccgcctctg atggatttga tgagcaagtc      240
141 agggaactca gggagagagc aaaggagatt cctgatgatt attttgttgt tttggttgga      300
143 gacatgataa cggaagaagc ccttcccact tatcaaaca tgctgaatac cttggatgga      360
145 gttcgggatg aaacaggtgc aagtcctact tcttgggcaa tttggacaag ggcattgact      420
147 gcggaagaga atagacatgg tgacctctc aataagtatc tctacctatc tggacgagtg      480
149 gacatgaggg aaattgagaa gacaattcaa tatttgattg gttcaggaat ggatccacgg      540
151 acagaaaaca gtccatacct tgggttcac tatacatcat tccaggaaaag ggcaaccttc      600
153 atttctcatg ggaacactgc ccgacaagcc aaagagcatg gagacataaa gttggctcaa      660
155 atatgtggtg caattgctgc agatgagaag cgccatgaga cagcctacac aaagatagtg      720
157 gaaaaactct ttgagattga tcctgatgga actgttttgg cttttgctga tatgatgaga      780
159 aagaaaattt ctatgcctgc acacttgatg tatgatggcc gagatgataa tctttttgac      840
161 cacttttcag ctgttgcgca gcgtcttgga gtctacacag caaaggatta tgcagatata      900
163 ttggagttct tgggtggcag atggaagggt gataaactaa cgggcctttc agctgaggga      960
165 caaaaggctc aggactatgt ttgtcggtta cctccaagaa ttagaaggct ggaagagaga     1020
167 gctcaaggaa gggcaaagga agcaccacc atgcctttca gctggatttt cgataggcaa     1080
169 gtgaagctgt ag                                     1092

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172 <210> SEQ ID NO: 3

173 <211> LENGTH: 34

174 <212> TYPE: DNA

C--> 175 <213> ORGANISM: Artificial

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```

177 <220> FEATURE:
178 <221> NAME/KEY: misc_feature
179 <223> OTHER INFORMATION: PCR primer; sequence flanking unique XbaI site at the 5' end
of t
180     he open reading frame
183 <400> SEQUENCE: 3
184 gtgagcggat aacaatttca cacagtctag aaat          34
187 <210> SEQ ID NO: 4
188 <211> LENGTH: 72
189 <212> TYPE: DNA
C--> 190 <213> ORGANISM: Artificial
192 <220> FEATURE:
193 <221> NAME/KEY: misc_feature
194 <222> LOCATION: (56)..(57)
195 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n"
indicates
196     the presence of either C, A, T or G at that nucleotide position
199 <400> SEQUENCE: 4
WJ> 200 ccaaattgcc caagacgtcg gacttgcacc tgtttcatcc cgaactccat ccaamnnatt      60
202 cagcattggt tg          72
205 <210> SEQ ID NO: 5
206 <211> LENGTH: 31
207 <212> TYPE: DNA
C--> 208 <213> ORGANISM: Artificial
210 <220> FEATURE:
211 <221> NAME/KEY: misc_feature
212 <223> OTHER INFORMATION: PCR primer
215 <400> SEQUENCE: 5
216 gaaacagggtg caagtccgac gtcttgggca a          31
219 <210> SEQ ID NO: 6
220 <211> LENGTH: 26
221 <212> TYPE: DNA
C--> 222 <213> ORGANISM: Artificial
224 <220> FEATURE:
225 <221> NAME/KEY: misc_feature
226 <223> OTHER INFORMATION: PCR primer
229 <400> SEQUENCE: 6
230 gttttctgtc cgcggatcca ttctgt          26
233 <210> SEQ ID NO: 7
234 <211> LENGTH: 34
235 <212> TYPE: DNA
C--> 236 <213> ORGANISM: Artificial
238 <220> FEATURE:
239 <221> NAME/KEY: misc_feature
240 <223> OTHER INFORMATION: PCR primer
243 <400> SEQUENCE: 7
244 gtgagcggat aacaatttca cacagtctag aaat          34
247 <210> SEQ ID NO: 8
248 <211> LENGTH: 30
249 <212> TYPE: DNA
C--> 250 <213> ORGANISM: Artificial

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252 <220> FEATURE:
253 <221> NAME/KEY: misc_feature
254 <223> OTHER INFORMATION: PCR primer
257 <400> SEQUENCE: 8
258 cacgaggccc ttctgtcttc aagaattctc 30
261 <210> SEQ ID NO: 9
262 <211> LENGTH: 28
263 <212> TYPE: DNA
C--> 264 <213> ORGANISM: Artificial
266 <220> FEATURE:
267 <221> NAME/KEY: misc_feature
268 <223> OTHER INFORMATION: PCR primer
271 <400> SEQUENCE: 9
272 ttgataagtg ggaagggctt cttccgtt 28
275 <210> SEQ ID NO: 10
276 <211> LENGTH: 66
277 <212> TYPE: DNA
C--> 278 <213> ORGANISM: Artificial
280 <220> FEATURE:
281 <221> NAME/KEY: misc_feature
282 <222> LOCATION: (41)..(43)
283 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n"
indicates
284 the presence of either C, A, T or G and in which "k" indicates t
285 he presence of either T or G.
288 <220> FEATURE:
289 <221> NAME/KEY: misc_feature
290 <222> LOCATION: (32)..(34)
291 <223> OTHER INFORMATION: PCR primer is degenerate oligonucleotide in which "n"
indicates th
292 e presence of either C, A T, or G at that nucleotide position and
293 in which "k" indicates either T or G
296 <220> FEATURE:
297 <221> NAME/KEY: misc_feature
298 <222> LOCATION: (44)..(46)
299 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n"
indicates
300 the presence of either C, A, T, or G at that nucleotide position
301 and in which "k" indicates the presence of either T or G.
304 <400> SEQUENCE: 10
WJL> 305 aacggaagaa gcccttccca cttatcaaac annkctgaat nnknknkgatg gagttcggga 60
307 tgaaac 66
310 <210> SEQ ID NO: 11
311 <211> LENGTH: 26
312 <212> TYPE: DNA
C--> 313 <213> ORGANISM: Artificial
315 <220> FEATURE:
316 <221> NAME/KEY: misc_feature
317 <223> OTHER INFORMATION: PCR primer
320 <400> SEQUENCE: 11
321 tccattcctg aaccaatcaa atattg 26
324 <210> SEQ ID NO: 12

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RAW SEQUENCE LISTING

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TIME: 15:51:00

Input Set : A:\ES.txt

Output Set: N:\CRF3\01102002\J017145.raw

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325 <211> LENGTH: 70
326 <212> TYPE: DNA
C--> 327 <213> ORGANISM: Artificial
329 <220> FEATURE:
330 <221> NAME/KEY: misc_feature
331 <222> LOCATION: (22)..(24)
332 <223> OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n"
indicates
333     the presence of either C, A, T or G at that nucleotide position
334     and in which "k" indicates the presence of either T or G at that
335     nucleotide position.
338 <220> FEATURE:
339 <221> NAME/KEY: misc_feature
340 <222> LOCATION: (28)..(30)
341 <223> OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n"
indicates
342     the presence of either C, A, T or G at that nucleotide position
343     and in which "k" indicates the presence of either T or G at that
344     nucleotide position.
347 <220> FEATURE:
348 <221> NAME/KEY: misc_feature
349 <222> LOCATION: (49)..(51)
350 <223> OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n"
indicates
351     the presence of either C, A, T or G at that nucleotide position
352     and in which "k" indicates the presence of either T or G at that
353     nucleotide position.
356 <400> SEQUENCE: 12
W 357 ttgattggtt caggaatgga tnnkcggnnk gaaaacagtc cataccttnn kttcatctat      60
359 acatcattcc                                                                70
362 <210> SEQ ID NO: 13
363 <211> LENGTH: 30
364 <212> TYPE: DNA
C--> 365 <213> ORGANISM: Artificial
367 <220> FEATURE:
368 <221> NAME/KEY: misc_feature
369 <223> OTHER INFORMATION: PCR primer
372 <400> SEQUENCE: 13
373 gcaaaaagcca aaacggtacc atcaggatca                                     30

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VERIFICATION SUMMARY

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DATE: 01/10/2002

TIME: 15:51:01

Input Set : A:\ES.txt

Output Set: N:\CRF3\01102002\J017145.raw

L:9 M:259 W: Allowed number of lines exceeded, <130> FILE REFERENCE:
L:11 M:270 C: Current Application Number differs, Replaced Current Application No
L:11 M:271 C: Current Filing Date differs, Replaced Current Filing Date
L:175 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:3
L:190 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:4
L:200 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:4
L:208 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:5
L:222 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:6
L:236 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:7
L:250 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:8
L:264 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:9
L:278 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:10
L:305 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:10
L:313 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:11
L:327 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:12
L:357 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:12
L:365 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:13